

LXXI. THE COMBINATIONS OF HAEMOGLOBIN WITH OXYGEN AND CARBON MONOXIDE, AND THE EFFECTS OF ACID AND CARBON DIOXIDE.

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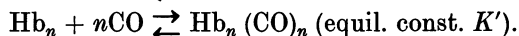
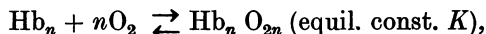
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A CRUCIAL test of any theory of the mode of combination of haemoglobin with oxygen and carbon monoxide, is to attempt to explain by means of it the remarkable relations discovered by Douglas, Haldane and Haldane [1912]. It is well known that the oxygen dissociation curve of blood follows the equation,

$$y = \frac{Kx^n}{1 + Kx^n}$$

where y is the relative degree of saturation of the haemoglobin, x the concentration of oxygen, and K a constant. This represents a tilted S-shaped curve which—whether by chance or not—certainly fits the observed points with a high degree of accuracy, and under a wide variety of conditions. This curve is affected by the presence of acid or CO_2 : according to Henderson [1920] and Adair [1921] the value of $1/K$ is a linear function of the pressure of the CO_2 , n remaining constant, the actual effect on the curve being to stretch it in the direction of x , without changing its shape. The first remarkable fact [Douglas, Haldane and Haldane, 1912, p. 287] is that the dissociation curve of CO is precisely similar to that of O_2 , being an S-shaped curve with the same value of n , and showing qualitatively and quantitatively the same effect of CO_2 . These facts can be deduced from the laws of mass action, if we assume that the reactions are governed by the equations,



These equations however lead immediately to a paradox. If we supposed them to apply at the same time, viz. to haemoglobin in contact with a gas containing both O_2 and CO in sufficient quantities to saturate the haemoglobin completely, and if z be the proportion of the haemoglobin combined with CO, we should find,

$$z = \frac{K'/K (\text{CO}/\text{O}_2)^n}{1 + K'/K (\text{CO}/\text{O}_2)^n}$$

which, if the concentration of oxygen in the gas be kept constant, represents the CO-dissociation curve of blood in the presence of constant excess of oxygen,

and is an *S-shaped curve exactly similar in all respects to those given by O₂ and CO separately*. This however is contrary to the facts, as shown in the paper referred to (p. 278): the actual curve is a smooth rectangular hyperbola, with no trace of an inflexion in it, and completely unaffected by the presence of CO₂. Clearly there is something wrong with the simple theory. Again, from the formulae as written down above, it is obvious that decreasing the concentration of oxygen will always liberate more haemoglobin to combine with CO, and so lead to a greater CO-saturation. This again is untrue: in the paper referred to [p. 291] it is clearly shown that below a certain limit decreasing the O₂ concentration *decreases* the amount of haemoglobin combined with CO, and indeed Haldane and Lorrain Smith [1897] observed the remarkable and paradoxical fact that the administration of CO might diminish the symptoms of anoxaemia in mice subjected to low oxygen pressures.

The affinity of CO for the haemoglobin of human blood at body temperature is some 246 times that of oxygen, and if we suppose (i) that, as regards saturating the haemoglobin (without respect to the nature of the gas with which it is saturated), a mixture of *a* parts of O₂ and *b* parts of CO is precisely similar in all respects to (*a* + 246*b*) parts of oxygen, and (ii) that in the resulting partly saturated and partly unsaturated mixture the saturated part is divided between O₂ Hb and CO Hb in the ratio of *a* : 246*b*, then all the facts established by Douglas, Haldane and Haldane can be explained. If *y* be the saturated fraction of the haemoglobin we then have

$$y = \frac{K(O_2 + 246CO)^n}{1 + K(O_2 + 246CO)^n}$$

of which a quantity

$$\frac{y(O_2)}{(O_2 + 246CO)}$$

is saturated with oxygen, and a quantity

$$\frac{y246(CO)}{(O_2 + 246CO)}$$

is saturated with CO. If *y* = 1, *i.e.* if the haemoglobin be fully saturated, it is clear that the CO-dissociation curve under constant excess O₂-concentration, is a rectangular hyperbola, and Douglas, Haldane and Haldane [1912, p. 291] showed that the paradoxical effect of diminished O₂-concentration producing a diminished CO-saturation could be explained, quantitatively and qualitatively, by the same hypothesis. The following statement of the phenomenon may make plainer what appears to be a very crucial point in the theory of haemoglobin combinations.

In Fig. 1 *AB* is the saturation of the haemoglobin with O₂ at O₂-pressure *OB*. CO gas is added to the oxygen at constant volume. The "equivalent oxygen pressure" (O₂ + 246 CO), is thus raised by $\frac{1}{3}$ (say) to *OD* by adding CO in pressure *BD*/246, thereby increasing the total saturation with gas to *CD*. Of this total saturation there are—according to the hypothesis—three parts of O₂ Hb corresponding to *OB*, and one part of COHb corresponding to *BD*, so that the oxygen saturation is now $\frac{3}{4}$ of *CD*, which, since *CA* is the steep

part of the curve, may be much larger than AB . Consequently, by adding an appropriate quantity of CO to the oxygen, the O_2 -saturation has been considerably *increased*. We will consider here only the theoretical bearings of this curious and paradoxical result.

The hypothesis described above has not yet been put into physico-chemical terms. By a comparatively simple extension, however, of the physico-chemical theory which gives the S-shape to the O_2 -dissociation curve, and the so-called "Hill's equation,"

$$y = \frac{Kx^n}{(1 + Kx^n)},$$

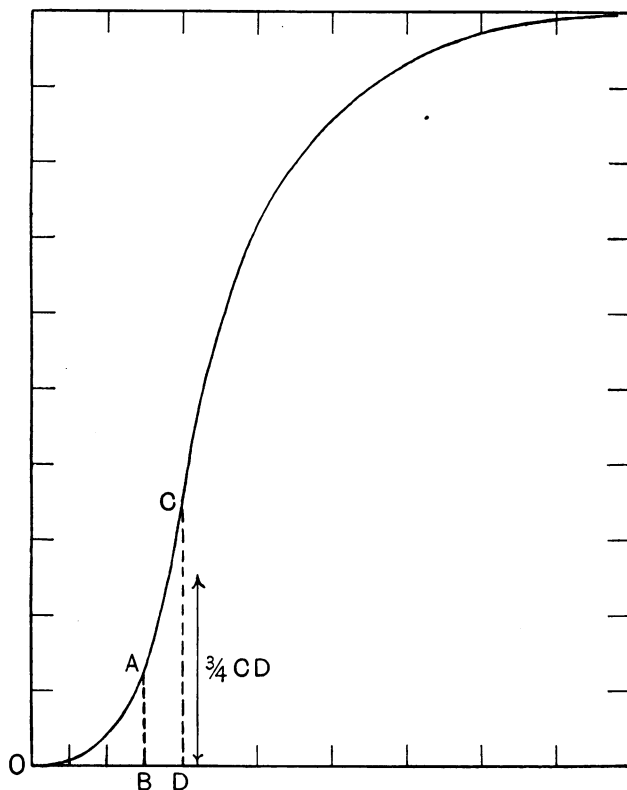
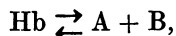


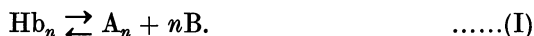
Fig. 1.

it is possible to satisfy all the relations described above, and thus to include the behaviour of O_2 and CO, separately and together, in one simple physico-chemical scheme. Let us assume that haemoglobin is a slightly dissociated compound of the iron-containing and the protein molecules, according to the equation,



where A is the protein and B is the iron-containing part. This assumption is not new, since it was a central one in Bohr's [1903] theory of the combinations of haemoglobin.

Let us assume further that in the presence of salts the osmotic pressure of the complex Hb, and of the simple protein A, is reduced to $1/n$ of its value calculated on the assumption that one molecule of haemoglobin contains one atom of iron. This assumption, as will be shown in a forthcoming paper, can be verified experimentally. It is not necessary to assume that this reduction of osmotic pressure is due to aggregation of the molecules, as was previously done [Hill, 1910]. Any cause whatever which reduces the osmotic pressure will give an S-shaped dissociation curve, as can be shown by rigid thermodynamical reasoning. The iron-containing molecule B, being small, we should expect to be unaffected in this respect, so that in the presence of salts we may assume that the above equation runs,



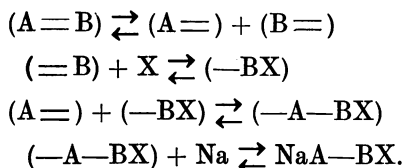
Let us now assume that the combination of haemoglobin with a gas X (either O_2 or CO) takes place only between molecules in the dissociated form, *i.e.* not between Hb and the gas, but between free B and the gas, according to the scheme,



the gas-saturated iron-containing molecule then recombining with the protein molecule according to the scheme,



There would seem to be some evidence for this. Oxyhaemoglobin is known to be a stronger acid than reduced haemoglobin, which suggests that in reduced haemoglobin the protein molecule and the iron-containing molecule are combined by two separate linkages, while in oxyhaemoglobin the two molecules are combined by only one linkage, the spare linkage of the protein requiring sodium or some other positive ion and the spare linkage of the iron-containing molecule requiring a gas, for its saturation. Thus the combinations would appear to run as follows:



Now if the process of combination of haemoglobin with a gas were as suggested, the recombination of the protein with the gas-saturated iron-containing parts might be expected to take place quite independently of the nature of the gas with which the latter was combined. In other words, in equation (III) the equilibrium constant is independent of the nature of the gas X, and is concerned only with the fact that one of the affinities of B has been satisfied. In equations (I) and (III), assuming the degree of dissociation of haemoglobin to be very small, if the proportion of saturated haemoglobin $(\text{HbX})_n$ be

called y , and the proportion of unsaturated haemoglobin Hb_n be called $(1 - y)$, we have, if K_1 and K_3 be the constants of reactions (I) and (III),

$$y = K_3(A_n)(BX)^n, \quad \dots(IV)$$

$$(1 - y) = K_1(A_n)(B)^n. \quad \dots(V)$$

Assume first that the gas X is O_2 only. Then equation (II) gives

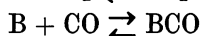
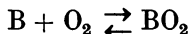
$$(BX) = K_O(B)(X),$$

where K_O is the equilibrium constant of the O_2 reaction. Combining this with (IV) and (V) we find,

$$\frac{y}{1-y} = \frac{K_3}{K_1}(K_O)^n(X)^n = K(X)^n \text{ say}$$

which is the ordinary equation to the O_2 -dissociation curve. Similarly, if the gas X be CO only, we obtain identically the same equation with another constant K . Furthermore, CO_2 may be assumed (see below) to act upon the combination by capturing some of the Na satisfying the spare linkage on the protein molecule, and so reducing the value of K_3 . Its effect therefore would be identical in the two cases of O_2 and CO, as observed.

Finally, if the gas X be a mixture of O_2 and CO, the equation (II) becomes,



with the result,

$$(BO_2) = K_O(B)(O_2)$$

$$(BCO) = K_{CO}(B)(CO)$$

from which,

$$(BX) = (BO_2) + (BCO) = (B)[K_O(O_2) + K_{CO}(CO)].$$

Combining this with (IV) and (V) we find,

$$\frac{y}{1-y} = \frac{K_3}{K_1}[K_O(O_2) + K_{CO}(CO)]^n.$$

Thus the total amount of haemoglobin saturated with gas is

$$y = \frac{K_3/K_1[K_O(O_2) + K_{CO}(CO)]^n}{1 + K_3/K_1[K_O(O_2) + K_{CO}(CO)]^n}$$

of which a fraction,

$$K_O(O_2)/[K_O(O_2) + K_{CO}(CO)]$$

will be saturated with O_2 , and a fraction,

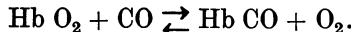
$$K_{CO}(CO)/[K_O(O_2) + K_{CO}(CO)],$$

with CO. These relations are identical with those shown above (p. 578) to satisfy all the observed facts. Further, with regard to the action of CO_2 , if the amount of O_2 and CO be sufficient to saturate the haemoglobin completely, it is clear that the rectangular hyperbola giving the distribution of the haemoglobin between CO and O_2 is affected only by the constants K_O and K_{CO} , and not at all by the constants K_3 and K_1 . But the action of CO_2 , on this theory, lies in its tendency to seize upon the Na satisfying the spare linkage of the gas-saturated haemoglobin complex, *i.e.* in its effect on the constant K_3 : thus the presence of CO_2 should have no effect on the relative distribution of

saturated haemoglobin between CO and O₂, which depends only upon the constants K_O and K_{CO} . This again is in accordance with observation.

There are certain other facts which, although indirectly, provide independent confirmation of the theory. There is no doubt that the combination between the protein and the iron-containing part, is a very loose one, and it is known that chemical agents (*e.g.* potassium ferricyanide), or bacteria, or a high temperature, act much more readily on haemoglobin when uncombined with gas, than when combined, and much more readily on oxy- than on CO-haemoglobin [see *e.g.* Hartridge, 1912, 2]. One would expect the active agent to work more readily on dissociated molecules. But the presence of a large proportion of unsaturated Hb would lead (see equation (I)), by the laws of mass action, to a large proportion of free dissociated molecules. The presence of O₂ would eliminate these molecules by combination, as in equation (II). The presence of CO however, even at low pressures, would eliminate them much more effectively, since a small breakdown of the gas-combined compound would immediately and enormously raise the effective combining pressure of the CO, leading to an almost complete absence of the dissociated molecules of haemoglobin.

A further point of interest arises in connection with the effect of temperature. Hartridge [1912, 1] found a relatively small, but definite, effect of temperature upon the position of equilibrium of the reaction,



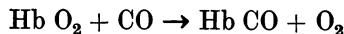
Taking his figures, and employing the equation

$$K = K_0 e^{-q(T-T_0)/2TT_0}.$$

to determine the heat of reaction, we find

$$q = 5240 \text{ calories per g. molecule.}$$

This is some 19 % of the value found by Barcroft and Hill [1910], by the same method, for the heat of reaction of one gram-molecule of oxygen with dialysed haemoglobin, and about 50 % of the value similarly found in hitherto unpublished experiments by Brown and Hill in the case of whole blood. Recent experiments however by Hartridge [1921] have shown that his earlier observations were in error, by not allowing for a natural shift of the α -band with temperature, and suggest that the effect of temperature on the position of equilibrium of O₂ and CO with Hb is really *nil*, or practically *nil*, a conclusion which confirms an earlier observation of Haldane and Lorrain Smith. In that case the heat of reaction of

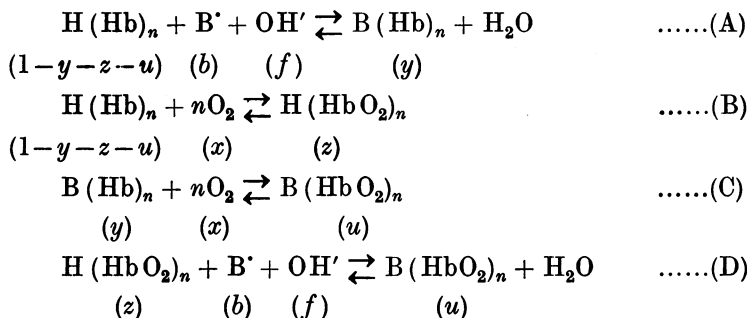


is very small, or nothing. Thus, even though CO has hundreds of times the affinity for Hb that O₂ has, its heat of reaction is almost exactly the same: this makes all the more likely the hypothesis that in the recombination of the protein with the gas-saturated iron-containing molecule no effect is exerted by the actual chemical nature of the gas, but only by the fact that a

free affinity is satisfied; combination with either gas has reduced the total energy of the molecule by identically the same amount, and the protein is indifferent as to which gas has done it.

The effects of acids and CO₂ on the dissociation curve.

It has been known for some time, chiefly as the result of the work of Barcroft, that the addition of acid to blood has a large effect on the dissociation curve, and recently Adair and Henderson have shown empirically that $1/K$ is a linear function of the CO₂ pressure. It is possible to deduce these interesting and important facts from the theory as follows: we suppose that the haemoglobin in blood is $H.(Hb)_n$, a very weak acid, while the oxyhaemoglobin is $H.(HbO_2)_n$, a stronger acid [Christiansen, Douglas and Haldane, 1914]. The following equilibria then co-exist inside the corpuscle (small letters represent concentrations, and B represents alkali, Na or K, inside the corpuscle):



From these equations we have,

$$\text{From (A),} \quad \frac{y}{1-y-z-u} = k_1fb.$$

$$\text{From (C),} \quad u/y = k_3x^n.$$

$$\text{From (D),} \quad u/z = k_4fb,$$

where k_1 , k_2 , k_3 , and k_4 are the equilibrium constants of the several reactions.

From these we can deduce that,

$$\frac{z+u}{1-(z+u)} = x^n k_3 \frac{(1+1/k_4fb)}{(1+1/k_1fb)}.$$

But $(z+u)$ is the O₂-saturated fraction of the haemoglobin, $[1-(z+u)]$ the unsaturated fraction, so that this equation is the same as the ordinary "Hill's equation" of the dissociation curve, with a K equal to $k_3(1+1/k_4fb)/(1+1/k_1fb)$.

$$\text{Hence,} \quad \frac{1}{K} = \frac{1}{k_3} \frac{(1+1/k_1fb)}{(1+1/k_4fb)}.$$

Now oxyhaemoglobin is a much stronger acid than reduced haemoglobin, or in other words, k_4 is much greater than k_1 . But, if k_4 be large, $1/k_4fb$ will be small, and we may neglect it in comparison with the other terms. Assuming that we may neglect it we obtain immediately,

$$\frac{1}{K} = \frac{1}{k_3} (1 + 1/k_1fb).$$

Let k be the dissociation constant of water: then $f = (\text{OH}') = k/\text{C}_\text{H}$: hence

$$\frac{1}{K} = \frac{1}{k_s} (1 + \text{C}_\text{H}/kk_1b).$$

Thus $1/K$ depends upon k_s , k , k_1 , which are chemical constants, independent of everything presumably except temperature, and upon C_H/b , the ratio of the hydrogen to the basic ion concentration. Thus $1/K$ will be increased, and the dissociation curve shifted to the right, by an increase in the hydrogen ion concentration of the contents of the red blood corpuscle. This agrees with the experimental facts. A study of the actual quantities involved shows that C_H/kk_1b is usually so large compared with 1 that the equation may be written, without serious error,

$$1/K = \text{C}_\text{H}/kk_1k_3b.$$

This also agrees with the facts. Barcroft and Peters [1914], and Donegan and Parsons [1919], have shown that the relation between $\log K$ and the P_H ($-\log \text{C}_\text{H}$) of the plasma is a straight line (the so-called "P-B line"). This means that $1/K$ is proportional to some power of C_H , and since this straight line is usually inclined at 45° to the axes this power is usually the first: thus $1/K$ is proportional to $(\text{C}_\text{H})^m$ where m is generally unity. This agrees with the above deduction. If we suppose the haemoglobin molecule to be capable of reacting with more than one molecule of base, *e.g.* according to the scheme,



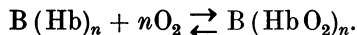
then $1/K$ will be found to depend not upon the first but upon some other power of the hydrogen ion concentration: this may account for "P-B lines" inclined at an angle other than 45° to the axes. According to the equation $1/K$ varies inversely with b the concentration of basic ions. Thus it is not the hydrogen ion concentration alone which determines the dissociation curve: it is *the ratio, inside the corpuscle, of the hydrogen ion to the basic ion concentration. These two ions must be regarded as competing with one another for possession of the haemoglobin.* One individual may have a higher Na' or K' concentration inside his corpuscles than another, so that at a given C_H their dissociation curves will be different: this may explain certain well-known differences between individuals or species.

In the presence of CO_2 the C_H *outside* the corpuscle is given by the relation $\text{C}_\text{H} = \alpha (\text{H}_2\text{CO}_3)/(\text{HCO}_3')$, where (H_2CO_3) is the concentration of dissolved CO_2 in the plasma, (HCO_3') that of bicarbonate ions, and α is the dissociation constant of carbonic acid. If we assume that the C_H of the plasma is the same as, or is proportional to, the C_H of the corpuscular contents, we may substitute this value of C_H , or a number proportional to it, in the above equation, so obtaining,

$$\frac{1}{K} = \frac{1}{k_s} [1 + \alpha (\text{H}_2\text{CO}_3)/kk_1b (\text{HCO}_3')].$$

Thus, on the theory, $1/K$ is a linear function of the CO_2 pressure, in direct agreement with Adair's [1921] experimental results. Moreover, this form of

the equation gives some meaning to the constants. The constant $1/k_3$ (Adair's constant b) is the inverse of the equilibrium constant of the reaction



Being the constant of a definite chemical reaction we should expect to find it invariable except for a change of temperature. Adair finds it practically unaffected, in general, by dyspnoea. On the other hand the constant

$$a/kk_1b(HCO_3')$$

(Adair's a/b) is simply a certain constant divided by the product of the concentrations (i) of basic ions inside the corpuscle and (ii) of bicarbonate ions outside. In dyspnoeic cases Adair found a much higher value of his a , and about the same value of his b , *i.e.* a higher value of a/b . Thus, on the theory,

$$a/kk_1b(HCO_3')$$

is increased in dyspnoea. But a/kk_1 should be invariable (except for a change of temperature) being derived from the constants of definite chemical reactions. Hence any increase in Adair's a/b must be due to an increase in $1/b(HCO_3')$. But in dyspnoea we should expect HCO_3' , the amount of free dissociated bicarbonate present, to be reduced, and $1/b(HCO_3')$ to be increased. This agrees therefore with Adair's experimental results. Thus the increase, in dyspnoea, of the ratio a/b of Adair's constants a and b may be credited simply to a reduction in the amount of bicarbonate in the plasma, or possibly to a reduction in the concentration of basic ions inside the corpuscle: while the linear relation between $1/K$ and the CO_2 pressure is due to the combinations of reduced- and oxy-haemoglobin with the base which is being competed for simultaneously by the CO_2 . Thus the effects of CO_2 upon the dissociation curve of blood can be attributed simply to physico-chemical reactions between the haemoglobin regarded as an acid, the sodium bicarbonate, and the CO_2 . It would be interesting to pursue the subject further by an experimental investigation of the effects of temperature, etc., upon the several reactions involved.

SUMMARY.

A theory is suggested to explain:

- (a) the S-shaped dissociation curve of oxy-haemoglobin in the presence of salts, and the similar curve of CO-haemoglobin;
- (b) the identical effects of CO_2 upon these curves;
- (c) the rectangular hyperbola relating CO-saturation to CO-pressure, or O_2 -saturation to O_2 -pressure, in blood or haemoglobin fully saturated with a mixture of O_2 and CO;
- (d) the absence of any effect of CO_2 upon these curves;
- (e) the paradoxical relationship between CO-saturation and O_2 -pressure, or between O_2 -saturation and CO-pressure, in blood or haemoglobin only partly saturated with a mixture of O_2 and CO.

It is shown also that the effects of acid, and of CO_2 , upon the dissociation curve of blood, can be deduced from the hypothesis that the available alkali inside the corpuscle is competed for by the oxy-haemoglobin, the reduced haemoglobin, and the acid or CO_2 , the first being a far stronger acid than the second. The so-called "P-B" line (the relation between $\log K$ and P_{H}), and the Adair-Henderson line (the relation between $1/K$ and CO_2 pressure) are thereby explained. The constant $1/K$ is shown to depend upon the ratio of the hydrogen to the basic ion concentration inside the corpuscle. This may explain the differences in the dissociation curves of different individuals.

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